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Effect of Hormones on Eyelid Disjunction in the Rat

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Effect of Hormones on Eyelid Disjunction in the Rat

In the course of a study of the effects of testosterone propionate on body growth in the newborn rat, it was observed that testosterone is a factor influencing the opening of the eyelids.

In recent years, it has become increasingly evident that hormones exert an influence on the process of eyelid opening in the rat. Thus *Mulinos and Pomerantz* (1942) noted that desoxycorticosterone, administered in dosages not affecting body weight, had a stimulatory effect. Corticosterone and cortisone were shown by *Parmer* et al. (1951) to have a similar effect. Likewise thyroid hormone was found to hasten the separation of the eyelids in mice (*Hamburgh and Vicari*, 1957) while thiouracil retarded this process in rats (*Eayrs*, 1951).

Donaldson (1924) observed that in the same litter the eyelids of the females open before those of the male rats. Investigating factors which might govern eye opening, *Eayrs* (1951) reported that topical administration of testosterone propionate to the closed eyelid retarded eye opening among litters of mixed sex. *Eayrs* related this effect to a concomitant reduction in body growth.

This paper will report the experimental results gathered following the daily subcutaneous injection of the androgen.

Methods

A. Testosterone experiment: Fourteen litters of rats (Carworth strain) comprising 113 animals were employed. The animals were sexed at birth and wherever possible distributed within their own litter in a manner to provide the same number of each sex for the control as for the experimental groups.

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The littermates were kept together and under the care of their mother. From the day of birth, each experimental animal received 1.0 mg of testosterone propionate in 0.02 cc of cottonseed oil while their littermate controls received an equivalent volume of the cottonseed oil placebo. Injection sites were uniformly alternated so that no one site received the next injection until four days later. All subcutaneous injections were performed with a syringe controlled by a micrometer. Body weight was recorded periodically throughout the experiment.

From the time a litter reached 13 days of age, evidence for the separation of the eyelids was checked twice a day. At the first observation that one or more rats of a litter showed progress in the separation of their eyelids, the particular day was called "Day X". The disjunction progress and body weight were then noted for each rat in that litter and the results recorded. Thus each litter had a "Day X". In this manner it was possible to maintain uniform conditions for the members of a litter allowing only one major outside variable —the treatment. The "Day X" results were then grouped.

In an attempt to discern quantitatively the time delay in eyelid separation resultant from the androgen treatment, a litter of 8 males was subdivided into numerically equivalent control and treated groups. The process of disjunction was observed every 6 hours around the clock. Two points were allotted for a completely separated eyelid and one point for a partially opened eyelid. By totalling the points for each group of the litter at the time of each observation and then dividing this score by the final attainable score, it was possible to obtain a percentage for that particular time. As suggested by *Eayrs* (1951), "these percentages were then plotted against time, and the time at which each group of litter-mates gained 50% of the possible score was read off from the graphs".

B. Untreated litter: The disjunction progress of female as compared to their male littermates was noted periodically around the clock. A graph was constructed using the same grading system as explained above.

C. Thyroxine experiment: From the day of birth, four micrograms of L-thyroxine, dissolved in distilled water with the aid of a minimum amount of N/10 NaOH, were injected daily into each of four male rats. Their male littermates received subcutaneous injections of the placebo. Once the animals reached 12 days of age, eyelid disjunction progress was recorded several times a day and a graph was constructed.

The Chi-square method was employed wherever statistical analysis of the data is indicated.

Results

A. Table I shows that the daily subcutaneous injection of 1.0 mg of testosterone propionate retarded the opening of the eyelids in male rats (0.005 level of significance). A weaker tendency (0.1 [1] significance level) appears to exist among the females

[1] The "open" and "partially open" data were combined for the statistical analysis.

TABLE I

Effect of testosterone on eyelid disjunction in the male

Treatment	Number of animals in the group	Results		
		open	partially open	closed
a) Control	28	9	4	15
b) Testosteronetreated	28	2	1	25

TABLE II

Effect of testosterone on eyelid disjunction in the female

Treatment	Number of animals in the group	Results		
		open	partially open	closed
a) Control	29	14	6	9
b) Testosteronetreated	28	10	3	15

(Table II). Upon androgen treatment the female disjunction progress (b. Table II) resembles ($P = 0.01$) that of the untreated male (a. Table I).

On the basis of the rating system suggested above, the testosterone treated animals achieved the 50 percent rating 6½ hours later than their control littermates.

B. A sex difference (0.01 [1] level) is observed among the treated animals (compare b. Table I with b. Table II) although this difference is not as significant (0.1 [1] level) among the controls (compare a. Table I with a. Table II). The disjunction process in the untreated litter, followed every 6 hours around the clock, indicates that the males reach the 50 percent rating 6½ hours later than their female littermates. The majority of the control females reported in this paper showed final disjunction on the 15th postnatal day. Eyelid separation usually begins centrally with a pinpoint-size opening and then proceeds toward the canthi.

When analyzed, the body weights of the male experimental (average 19.9 gm) and control animals (average 21.0 gm) were not found to be significantly different; likewise there was also no significant difference in the body weights among the female experimental (average 20.2 gm) and control (average 20.0 gm)

[1] The "open" and "partially open" data were combined for the statistical analysis.

groups. Data compiled from 10 other litters indicate that 1.0 mg of testosterone propionate subcutaneously administered to newborn rats for a period of 15 days had no significant effect on body weight.

C. Daily thyroxine administration was followed by retardation in growth and a hastening of the opening of the eyelids. The treated animals reached the 50 percent rating 3½ days earlier than the controls.

Discussion

It appears that upon subcutaneous administration of testosterone propionate there is a delay in the separation of the eyelids.

Eayrs (1951) relates the delay in disjunction among testosterone treated animals to the reduction in growth. In variance with *Eayrs,* no decrease in body weight could be observed among the androgen treated animals; consequently it is assumed that testosterone causes the delayed disjunction independent of any effect on body weight. Indeed, cortisone (*Parmer* et al., 1951) and thyroid hormone have shown a precocious disjunction effect even though a concomitant reduction in growth was observed.

That testosterone treatment is followed by a reduction in adrenal weights is well substantiated in the literature (*Selye*, 1940; *Wider* et al., 1958). Testosterone's concurrent effect of increasing the adrenal content of vitamin B_{12} (*Wider* et al.) could occur due to decreased utilization of the vitamin in metabolic processes of the gland as a result of the decreased activity of that gland. Whether testosterone delays eyelid opening via decreasing adrenal activity or precipitates its effect directly is a matter for conjecture until further work is pursued.

It is to be noted that also estrogens (*Eayrs*) retard eyelid opening in the rat and are followed (*Bongiovanni* et al., 1959) by genital aberration in the human fetus when administered to the mother. In the latter incidence it is believed (*Deanesly*, 1961) that estrogen may act via the fetal adrenal glands.

It is conceivable that hormones likewise play a part in the formation and separation of the eyelids in the human fetus. Already in 1872, *Zehender and Manz* reported a case of Cryptophthalmos where the skin over the malformed eyes was continuous supposedly due to lack of eyelid formation; *Zehender* noted a hypospadias among other external genital variations. *Chiari* (1883),

Onishi (1912) in Japan, *Brodsky and Waddy* (1940) in Australia, and *Jauw* (1950) have likewise reported such genital variations occurring with Cryptophthalmos. Such genital aberrations have been shown to be due to abnormal sex hormone levels arising from the fetal glands, maternal glands or exogenous hormone-treatment of the mother (review, *Deanesly*, 1961). It is possible that abnormal sex hormones levels during a critical developmental period may likewise play a role in the pathogenesis of Cryptophthalmos.

Another lid anomaly is Ankyloblepharon adnatum (filiform and membraniform), a condition where at birth elastic strands behind the cilia and anterior to the orifices of the meibomian glands connect the margins of the eyelids. The bands are not mere epithelial remains of the previous lidfusion but are composed (*Wintersteiner*, 1898) of a central vascular connective tissue entirely surrounded by epithelium. Among various proposals is the theory that it is a congenital deviation due to a growth aberration (*Judge* et al., 1929; *Khanna*, 1957; *Lemtis and Neubauer*, 1959).

Both the latter and Cryptophthalmos are mentioned in this discussion in suggestion that hormones may be a factor in the genesis of these malformations. Parenthetically, it might be noted that *Fraser* et al. (1957) was repeatedly capable of producing cleft palate in new born mice cast by mothers treated with cortisone for 4 days during pregnancy and that *Nelson* (1957) was able to demonstrate congenital eyelid anomalies in the offspring of PGA-deficient mothers.

Evidence has also been accumulating (*Waardenburg* et al., 1961) which indicates that a hereditary element may likewise be concerned in these clinical eyelid-aberrations.

Summary

The effect of subcutaneous testosterone propionate on eyelid disjunction in 113 newborn rats has been studied. Delayed eyelid disjunction independent of a hormone effect on body weight has been demonstrated.

In addition, the acceleratory effect of L-thyroxine on eyelid separation in the rat is reported.

It is suggested that sex hormones may play a role in the teratology of Cryptophthalmos and Ankyloblepharon adnatum (filiform and membraniform).

Zusammenfassung

Der Effekt von subcutan verabfolgtem Testosteronpropionat auf die Augenlideröffnung bei 113 neugeborenen Ratten wurde untersucht. Es wurde eine verzögerte Augenlideröffnung nachgewiesen, ohne daß das Hormon einen Einfluß auf das Körpergewicht ausübte.

Darüber hinaus wird über den beschleunigten Effekt von L-Thyroxin auf die Lideröffnung bei Ratten berichtet.

Eine mögliche Rolle der Sexualhormone bei der Teratologie des Kryptophthalmus und des Ankyloblepharon adnatum (filiform und membraniform) wird diskutiert.

Résumé

L'effet de propionat de testostérone administré par voie souscutanée a été examiné chez 113 rats nouveau-nés. On a trouvé un retard de l'ouverture des paupières sans que l'hormone ne montre un effet sur le poids du corps.

En plus l'effet accélérateur sur l'ouverture des paupières de la L-thyroxine est demontré.

Le rôle possible des hormones sexuelles dans la tératologie du cryptophthalmus et de l'ankyloblepharon adnatum (filiforme et membraniforme) est discuté.

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